For the movement of the eye the contraction of the muscle opposing the movement has to be inhibited. In the torsional movement of the leaf it is found that the stimulation of one nerve causes in a contiguous nerve an opposite reaction. The nervous impulses of opposite signs reaching different flanks of the motile organ is thus of importance in the co-ordination of the resulting movement.

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The Ultra-Violet Absorption Spectra and the Optical Rotation of the Proteins of Blood Sera.

By S. Judd Lewis, D.Sc. (Tübingen), B.Sc. (London), F.I.C.

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The earlier part of this investigation was described in a paper entitled "The Ultra-Violet Absorption Spectra of Blood Sera," communicated by Sir William Ramsay, K.C.B., to the Royal Society in 1916 and published in the 'Proceedings' (series B, Vol. 89, pp. 327 to 335).

At the close of the paper, attention was directed to the inadequacy of the sector spectrophotometers then available, and reference was made to one of new design then under construction. In the meantime, a full description of this instrument has been published in a paper entitled "A New Sector Spectrophotometer" by the present writer, in the 'Transactions of the Chemical Society' (1919, vol. 115, pp. 312 to 319), together with figure and diagrams. With this instrument completely satisfactory results have been obtained, and with it most of the work now to be described has been done.

The earlier work had reference to serum as a whole; and as foreshadowed

in the paper cited, the later effort has been directed to a study of the proteid components of serum and their individual influence on the spectrum. For this, the Beit Research Fund Committee of the British Homœopathic Association have again given generously the necessary financial aid out of the funds placed at their disposal by Mr. Otto Beit for purposes of scientific research.

A search in the literature has failed to reveal any information on the subject, so that in all parts of the work it was necessary to break new ground. Two papers on " Ultraspectroscopic Studies in Blood Serum," one by T. Tadokoro and the other by T. Tadokoro and Y. Nakayama, appeared in America in the 'Journal of Infectious Diseases' for January, 1920 (vol. 26, pp. 1 to 7, and 8 to 15), and recall the subject matter of the first paper cited, but they do not in any way anticipate the present communication.

It has already been observed that the absorption band of serum is caused entirely or almost entirely by the proteins contained, and it became a matter for inquiry as to whether the albumin, pseudo-globulin and eu-globulin were similarly or variously absorbent of ultra-violet light.

The necessary preliminary to a spectroscopic examination of these components was to separate them in a pure state, and to devise means for determining the concentration of the solution employed. This proved an unexpectedly difficult task, partly because of the confused state of the information available and partly because of the necessity of employing solutions perfectly free from preservatives and other substances capable of affecting the spectrum-absorbing power of the solutions. In the end there was no alternative to relying on one's own discretion and to devising the details of the processes of separation and purification. Great care was exercised with a view to purity and constancy of product.

In formulating the processes, much attention was paid to those published by Hardy, Hartley and Haslam, but more particularly to the researches of Dr. Harriette Chick on the physical conditions which control the precipitation of the proteins of serum, published in 1913 and 1914 in the 'Biochemical Journal.'

Method of Separating the Proteins.

In the processes of separation ammonium sulphate was practically the only reagent employed. The purest qualities obtainable commercially were carefully tested for organic matter, as traces of this would affect the absorption spectrum, and the best specimens were selected.

The manner of procedure was in principle such as that usually followed, depending on suitable application of various concentrations of ammonium sulphate; but numerous details were carefully studied, and amongst these the

following may be mentioned. It was found desirable to employ fairly large quantities of material, such as two litres, of the clear serum and at each stage to work at first with solutions of ammonium sulphate, which were as precisely as possible of full, one-half or one-third saturation, and then, when required, to add small but known excesses. The albumin precipitates were dissolved in such a quantity of water as to produce a 14 or 16 per cent. solution, and reprecipitated, the first time by the addition of specially recrystallised ammonium sulphate, and on subsequent occasions by the aid of ammonium sulphate and a very small quantity of acetic acid, and left to stand over for some days to allow the precipitate to become micro-crystalline. With horse serum the best crystals were obtained after the fourth precipitation. With human serum, the particles did not form cystals, but they exhibited a well-defined uniformity of shape, suggestive of an approach to a radiate crystalline structure.

The mixed globulins were precipitated three times, and then separated from one another by dissolving in sufficient water to produce an approximately 2 per cent. solution, and precipitating by increasing the ammonium sulphate concentration to one-third saturation.

The pseudo-globulin was freed from accompanying eu-globulin by slightly increasing the ammonium sulphate to nearly 36 per cent. saturation and filtering, and then by adding four small equal quantities of saturated ammonium sulphate solution, and allowing to stand after each addition, so that the final concentration was nearly but not quite 37 per cent. saturated, with a view to removing any remaining eu-globulin. After filtration, the pseudo-globulin was reprecipitated by increasing the ammonium sulphate to one-half saturation, and collected for use.

The eu-globulin was reprecipitated by ammonium sulphate four times from dilute solutions. With ascitic fluid, a small amount of a brown jelly-like substance sometimes accompanied the eu-globulin. When this was the case, the impure eu-globulin was dissolved as completely as possible in a small quantity of water and then saturated ammonium sulphate solution was added, until a small but considerable precipitate was formed, stirred well and filtered. The jelly-like substance remained on the filter together with a small quantity (probably 20 or 30 per cent.) of the eu-globulin. The filtrate was then quite clear, and was treated as an ordinary solution of eu-globulin. Subsequent precipitations usually gave no trouble.

Optical Rotation of the Proteins.

It was at first assumed that each protein would have a constant rotatory power, and that observations of the specific rotations would settle the question

of purity. However, the literature of the subject revealed the widest disparity of figures for the same protein, and as reliable data did not appear to be available, recourse was had to determining the concentrations of the solution by chemical means and to taking advantage of the opportunity which the principal work afforded of determining the specific rotations of the several proteins. In view of the separations having been carried out with such thoroughness, the figures should be fairly accurate and reliable.

There is probably a normally definite specific rotation for each of the globulins, although experimental results do not favour this view with regard to the albumins. On the other hand, one must take into consideration such cases as that of the pseudo-globulin from specimen No. 201. The specific rotation was determined twice on two entirely independent solutions of different concentration, the concentrations having to be ascertained separately by chemical means. The two results are $-43{\cdot}26°$ and $-43{\cdot}82°$, whereas the adopted figure for other pseudo-globulins is $-46°$. This seems to show that if there is a normal value, there are specimens having abnormal values. A study of the figures as a whole leads to the conjecture that a given specimen may be pure in the chemical sense, but consist of a mixture of optical isomers of the protein. A closer examination of the data reveals many irregularities not apparent at first sight. For example, the two most recent and best specimens of pseudo-globulin from the horse had the specific rotations $-52{\cdot}06°$ and $-52{\cdot}17°$, while an earlier specimen gave $-49{\cdot}50°$. Corresponding specimens of human origin gave $-43{\cdot}5°$, $-46{\cdot}97°$, $-47{\cdot}66°$, and $-45{\cdot}35°$, the first three having been separated from ascitic fluid and the last from normal serum. There is thus exhibited a well-marked differentiation between the rotation of the human, which may be taken as $-46°$, and that of the horse, which may be taken as $-52°$.

Similarly with eu-globulin: the figures for the two best and most recent specimens of horse are $-43{\cdot}03°$ and $-43{\cdot}04°$, and for an earlier one $-40{\cdot}98°$. The human gave $-50{\cdot}24°$, $-49{\cdot}12°$, $-47{\cdot}13°$, and $-47{\cdot}89°$, the first three referring to the protein separated from ascitic fluid and the last from normal serum.

From these one may adopt $-43°$ for eu-globulin from horse and $-48°$ for the human.

With albumin the results fluctuated considerably. For horse $-57{\cdot}40°$ is the only figure available. For human, $-65{\cdot}36°$, $-64{\cdot}43°$, $-55{\cdot}05°$, $-59{\cdot}14°$, $-50{\cdot}58°$, $-54{\cdot}83°$, the first four referring to albumin separated from ascitic fluid, and the last two to that from normal serum.

The rotations were observed with solutions containing a little ammonium sulphate. By experiment it was ascertained that the change of rotation

on varying the concentration of the ammonium sulphate is so small as not appreciably to affect the specific rotations found. The effect under the prevailing conditions of experiment is not likely to be greater than $\pm 0{\cdot}2$ on the specific rotation.

Spectrophotometry of the Solutions.

Strong solutions of the proteins were obtained by dissolving the purified precipitates described above in water, and their concentrations were ascertained by determining the total solids and the ammonium sulphate in the solution, and taking the difference as protein. This method gave constant and apparently satisfactory results. In every case the work was done in duplicate, and sometimes in triplicate.

The strong solution was polarised with the object of determining the specific rotation, and suitably diluted with distilled water for use in the spectrophotometer. The concentrations found to work best, that is, such as to exhibit a well-developed band, have been found to be 0·08 per cent. for the albumin, 0·04 per cent. for the pseudo-globulin and for the eu-globulin.

The strengths of the solutions were estimated approximately by means of the polarimeter for immediate use, and corrected later when the chemical figures became available.

The dilution was filled into a 2-cm. observation tube fitted with quartz ends, and a second tube was filled with a solution of ammonium sulphate of approximately the same strength as was the protein solution with reference to this salt: usually this was obtained by diluting saturated ammonium sulphate solution 150 times. The latter tube was used as a blank in the one path of the spectrophotometer, so that the observations made with the tube of protein solution placed in the other path express the spectrum-absorbing effect of the protein only.

The process of spectrophotometry was conducted in the manner indicated in the paper first cited, with all the refinements described in the paper dealing with the new instrument. The series of photographs for each experiment extended over three plates, making a series of fifty-four in all.

The absorption curve is plotted with extinction coefficients as ordinates and wave-lengths as abscissæ. The extinction coefficient is calculated on a 1-cm. layer of a 0·1 per cent. solution of the protein, which, according to Beer's law, is the same as that on a 0·1-mm. layer of a 10 per cent. solution. This corresponds with the protein concentration of serum as nearly as decimal figures permit; serum contains about 8 per cent of proteins. Hence the curves approximate in their values to those already described with reference to serum itself. In order to correlate the two sets of values, either the

extinction coefficients of serum must be multiplied by 1·25 or those of the proteins by 0·8.

Each of the protein curves is in general similar in form and character to that of serum (*loc. cit.*), which demonstrates that the band produced by serum is an expression of its protein-content, especially since serum deprived of its protein gives no such band (*loc. cit.*).

It now remains to consider the specimens employed and the curve for each protein in detail. In all, eleven specimens of serum were studied, six derived from the horse and five human. The primary object in employing horse serum was to ascertain the best conditions for separating and purifying the proteins, so that the human serum might afterwards be studied with greater confidence and certainty. The two or three earlier numbers amongst the horse specimens may therefore be regarded as practice numbers, and to that extent the figures for these must be held as less reliable.

The horse serum was as nearly as possible strictly normal, as the first three specimens were supplied as such from a physiological laboratory; and the three later specimens were derived from animals slaughtered for use as human food. The serum was mixed with an equal volume of saturated ammonium sulphate solution within 24 hours of the slaughter of the horse.

The last of the specimens of human serum (No. 205) was declared to be strictly "normal," and was from a case of cerebral hæmorrhage. The other four were selected specimens of ascitic fluid. Each one was quite clear and had the appearance of good serum: No. 200 being the least satisfactory, although that was good.

The three later specimens of horse serum and those of ascitic fluid were all sufficiently large. That of normal human serum was smaller, namely, 250 c.c.; but in this case, by careful manipulation, satisfactory separation and purification of the three proteins were effected.

The experimental data are collated in Tables I to VI, in which the specific rotations are repeated for the sake of easy reference. The results are graphically displayed in the accompanying curves, which have been arranged in two groups, namely, the three proteins from horse serum in the one, and those of human origin in the other. This is convenient, because the curves for the two pseudo-globulins are so very nearly alike that they may be regarded as the one a replica of the other.

The values brought together in the Tables for study and comparison are (*a*) the extinction coefficient at the head of the absorption band at a wave-length of about 2800; (*b*) the extinction coefficient at the point in the band where the light-absorbing power is least at a wave-length of about 2500, that is, in the depression of the curve; (*c*) the difference between (*a*)

and (*b*), or the "amplitude" of the band, which shows considerable regularity, and appears to be significant; (*d*) the wave-length of the region of greatest absorption in the band, that is, at the head; (*e*) the wave-length of the point of least absorption in the band, that is at the foot of the curve in the depression.

Table I.—Pseudo-Globulin from the Horse.

Specimen number.	Extinction coefficient.			Wave-length.		Specific rotation.
	Of head at 2800.	Of foot at 2500.	Difference or amplitude.	Head.	Foot.	
						°
193	1·12	0·43	0·69	2790	2510	
194	1·31	0·52	0·79	2800	2510	
197	1·18	0·46	0·72	2780	2518	
198	2·01	1·41	0·60	2780	2530	−49·50
199	1·29	0·57	0·72	2800	2540	−52·06
203	1·18	0·46	0·72	2790	2500	−52·17
Adopted	1·19	0·47	0·72	2790	2520	−52

Table II.—Pseudo-Globulin (Human).

Specimen number.	Extinction coefficient.			Wave-length.		Specific rotation.
	Of head at 2800.	Of foot at 2500.	Difference or amplitude.	Head.	Foot.	
						°
200 ascitic fluid	1·26	0·61	0·65	2750	2520	
201 „ „	1·35	0·63	0·72	2780	2528	(*a*) −43·26
						(*b*) −43·82
202 „ „	1·46	0·72	0·74	2790	2520	−46·97
204 „ „	1·40	0·67	0·73	2805	2525	−47·66
205 normal serum	1·28	0·58	0·70	2790	2520	−45·35
Adopted	1·35	0·63	0·72	2790	2521	−46

Observations on the Figures for Pseudo-globulin, Tables I and II.

Omitting the earlier numbers, 193 and 194, which represent the first efforts, and 200, which was not a very good specimen, the figures exhibit a remarkable regularity in the amplitude of the absorption curve as expressed by the difference in the extinction coefficients. With one exception (No. 198), they all lie between 0·70 and 0·74. Very little difference between the horse and human specimens is evident. The chief distinction is that the corresponding coefficients are a little higher for the human than for the horse.

One of the most striking features of the work is the discovery that pseudo-globulin has probably identically, certainly almost identically, the same form of absorption band of the same magnitude, whether it is of horse or human origin. This is good evidence of pseudo-globulin being a chemical entity.

Table III.—Eu-Globulin from the Horse.

Specimen number.	Extinction coefficient.			Wave-length.		Specific rotation.
	Of head at 2800.	Of foot at 2500.	Difference or amplitude.	Head.	Foot.	
						°
193	1·68	0·48	1·20	2800	2530	
194	—	0·53	—	—	2515	
197	—	—	—	—	—	
198	1·44	0·62	0·82	2760	2530	−40·98
199	1·09	0·62	0·47	2770	2530	−43·03
203	1·42	0·89	0·53	2780	2535	−43·04
Adopted	1·42	0·89	0·53	2775	2532	−43

Table IV.—Eu-Globulin—Human.

Specimen number.	Extinction coefficient.			Wave-length.		Specific rotation.
	Of head at 2800.	Of foot at 2500.	Difference or amplitude.	Head.	Foot.	
						°
200 ascitic fluid	1·37	0·71	0·66	2750	2630	−50·24
201 „ „	1·46	0·78	0·68	2770	2510	−49·12
202 „ „	1·39	0·67	0·72	2800	2540	−47·13
204 „ „	1·53	0·85	0·68	2805	2525	
205 normal serum	1·52	0·89	0·63	2790	2530	−47·89
Adopted	1·51	0·85	0·66	2795	2530	−48

Observations on the Figures for Eu-globulin, Tables III and IV.

The curves for the two eu-globulins are so similar in their general form and magnitude to those for pseudo-globulin as to leave no doubt of a close chemical relationship between the two groups, but the minor quantitative distinctions are too great and too well-defined to allow of their being regarded as mere varieties of the same substance.

At the present time, it is a matter of great interest to discover any fundamental differences between pseudo-globulin and eu-globulin. Dr. Harriette

Chick considers the two globulins to be the one a modification of the other.* The results of the present inquiry may, on the whole, be held to support this view, but they do certainly indicate also that there is some important difference between them, which is borne out by the two following considerations:—

(*a*) The amplitude figure is much greater for the pseudo-globulin than it is for the eu-globulin.

For pseudo-globulin this figure is 0·72 for both horse and human; for eu-globulin it is 0·53 for horse and 0·66 for human. Therefore, with both varieties, there is a marked difference between the two globulins.

Also, for both horse and human proteins, the curve for pseudo-globulin is very slightly broader than that for eu-globulin; this is so for the human more than for the horse.

(*b*) The intensity of the selective absorption is much greater for the eu-globulin than it is for the pseudo-globulin, as shown by the magnitudes of the extinction coefficients.

For horse, the figures for eu-globulin are 1·42 for the head and 0·89 for the foot, mean 1·16, against those for pseudo-globulin, which are 1·19 for the head and 0·47 for the foot, mean 0·83, giving an excess in favour of the eu-globulin of 0·33, or 39 per cent.

For human, the corresponding figures for eu-globulin are 1·51 for the head and 0·85 for the foot, mean 1·18, against those for pseudo-globulin, which are 1·35 for the head and 0·63 for the foot, mean 0·99, giving an excess in favour of the eu-globulin of 0·19, or 19 per cent.

In passing, it may be observed that both horse and human pseudo-globulin exhibit the same amplitude in the band, namely, 0·72, and that the bands for both horse and human eu-globulin have nearly the same mean values for the extinction coefficient, namely, 1·16 and 1·18.

Although the problem is not yet solved, it may be said that the spectroscopic phenomena are in harmony with the view expressed by Dr. Chick, that eu-globulin is a protein-lipoid complex resulting from the interaction of pseudo-globulin with a minute proportion of a lecithin.

Observations on the Figures for Albumin, Tables V and VI.

The extinction coefficients of albumin contrast strongly with those for either of the two globulins. The amplitude value is only 0·36 for the horse or 0·23 for the human, instead of about 0·7, which was found for the two globulins.

* 'Biochem. Journ.,' vol. 8, pp. 404–420 (1914).

Table V.—Albumin from the Horse.

Specimen number.	Extinction coefficient.			Wave-length.		Specific rotation.
	Of head at 2800.	Of foot at 2500.	Difference or amplitude.	Head.	Foot.	
						°
193	—	—	—	—	—	
194	0·55	0·32	0·23	2780	2520	
197	1·60	1·21	0·39	2780	2540	
198	1·42	0·97	0·45	2770	2540	−57·40
199	0·89	0·68	0·21	2770	2545	
203	1·19	0·84	0·35	2800	2550	
Adopted	1·20	0·84	0·36	2785	2545	

Table VI.—Albumin—Human.

Specimen number.	Extinction coefficient.			Wave-length.		Specific rotation.
	Of head at 2800.	Of foot at 2500.	Difference or amplitude.	Head.	Foot.	
						°
200 ascitic fluid	1·05	0·86	0·19	2770	2575	−65·36
201 „ „	0·67	0·37	0·30	2765	2540	−64·43
202 „ „	0·70	0·38	0·32	2795	2540	−55·05
204 „ „	0·58	0·33	0·25	2780	2540	−59·14
205 normal serum	*0·70	0·51	0·19	2780	2555	−50·58
	†0·66	0·45	0·21	2790	2538	−54·83
Adopted	0·68	0·45	0·23	2783	2540	

* 1st crop. † 2nd crop.

The distinction is not in the amplitude alone. The extinction coefficients at the head and foot of the curve in the horse series are fairly high, while in the human series they are exceptionally low, so that the horse albumin is well differentiated from the human albumin.

On tabulating the adopted figures for the amplitude of the band, the wave-length of the head and the wave-length of the foot, and extracting their means as shown in Table VII, one is impressed with the uniformity in the wave-length of the head, which is very nearly the same for the three proteins, and yet clearly not identical for pseudo-globulin (2790) and albumin (2784), while the two varieties of eu-globulin provide extremes at 2775 and 2795. The wave-lengths of the foot for the three proteins are also nearly the same, although again clearly differentiated: 2521 for pseudo-globulin, 2531 for eu-globulin, and 2543 for albumin. The differences are small, but there is no reason for doubting that they are real. The approximate

Table VII.—Comparative Observations on the Results.

	Amplitude.	λ for head.	λ for foot.
Pseudo-globulin—			
Horse	0·72	2790	2520
Human	0·72	2790	2521
Mean	0·72	2790	2521
Eu-globulin—			
Horse	0·53	2775	2532
Human	0·66	2795	2530
Mean	0·60	2785	2531
Albumin—			
Horse	0·36	2785	2545
Human	0·23	2783	2540
Mean	0·30	2784	2543

identity of the wave-lengths signifies the close similarity of chemical constitution for the three proteins, while the small differences, which are well substantiated so far as the present series of experiments goes, may possibly be significant of differences in the nature of the subsidiary groups present in the respective molecules. On the other hand, the amplitudes, which are respectively 0·72, 0·60 and 0·30, reveal differences which are too great to be lightly set aside.

It remains, then, that it is mainly in the magnitude of the extinction coefficients that the differences among these three proteins find expression. This has already received some attention in the comparison of eu-globulin with pseudo-globulin; but, with albumin, the divergence from the apparently well-defined pseudo-globulin is much greater, and not of quite the same order; and, further, there is no doubt as to horse albumin being quantitatively distinct from human albumin.

If the ratio of the adopted extinction coefficients for the two varieties of albumin be taken, we get

	Head of band.	Foot of band.	Amplitude.
$\frac{\text{Horse}}{\text{Human}}$	$\frac{1·20}{0·68} = 1·77$	$\frac{0·84}{0·45} = 1·87$	$\frac{0·36}{0·23} = 1·57$

or, if the figures for the two specimens of human albumin from "normal" serum alone be taken, we get

$$\frac{1·20}{0·68} = 1·77 \qquad \frac{0·84}{0·48} = 1·75 \qquad \frac{0·36}{0·20} = 1·80$$

This regular ratio for the several parts of the absorption curve would ordinarily signify a corresponding difference in the concentration of the substance in solution, but, inasmuch as the percentage concentration of the albumin was the same for all the solutions, both in the experiment and in the ultimate calculations, some other explanation must be found. The work of Kober* and others has shown that aromatic amino acids exhibit selective absorption, while the absorption spectra of several of the aliphatic amino acids and the simpler polypeptides exhibit no selective absorption, and that even 40 mm. layers of 0·05 per cent. solutions of some of the latter show no general absorption beyond a wave-length of 2500. In view of the way in which the proteids are built up of amino-acids and similar groups, some exerting selective absorption and some not, and of the difficulty in accounting for some of the properties of the proteins when viewed as chemical entities, it is not unreasonable to regard them as products resulting from an essentially physical association of substances comparable with, but not so chemical as, the association of a salt with its water of crystallisation. It may then be assumed that the aggregate composing human albumin may result from such a union of a substance comparable with that composing horse albumin with a substance or substances possessing no selective absorption. Such an hypothesis of physical association gains some support from the fact that five out of six of the amplitude values, 0·72, 0·72, 0·53, 0·66, 0·36, 0·20, are approximately simple multiples of 0·18, and from the view held by Prof. F. Gowland Hopkins† that even the apparently well-defined crystallised egg albumin is composed of several proteids, and that much the same may be said of serum albumin. It is conceivable that each of the proteins now studied is an aggregate resulting from the physical association of a proteid substance exhibiting selective absorption with various but definite proportions of such simpler bodies as those described above as exerting only general absorption. Had the results now recorded been anticipated, it would have been instructive to determine in each specimen the yield of phenylalanine, tryptophane and similar products exhibiting selective absorption, to see whether the proportion was correlated with the extinction coefficient or not. It would have given some indication of how far the association of the groups is physical or chemical.

Moreover, if all the curves be re-drawn so as to have the same amplitude, say 0·72 or 1·0, very little difference will be observed in their form. However, the two albumins would be distinguished from the globulins by the general absorption being slightly greater, extending to about 2450 at the

* 'J. Biol. Chem.,' vol. 22, pp. 433–441 (1915).

† 'J. Physiol.,' vol. 25, pp. 306–330 (1900).

extinction coefficient of the head of the band against 2420 for any of the globulins, and to this one may perhaps attribute the higher figure, 2543, for the wave-length of the foot of the curve in the albumins. The inference from this is that the general absorption of the simpler body in albumin is somewhat greater than it is in the globulins. It is interesting also to observe that the absorption curve of pseudo-globulin which has the greatest amplitude proved to be the most constant of all in the course of the experiments now described, and was the same whether for horse or man, and would thus appear to be a much more definite substance than either eu-globulin or albumin; it may even be a chemical entity.

On the other hand, it is possible that the differences between the absorption spectra of the proteins are essentially chemical in their significance, and then they are not capable of such simple explanation.

The Absorption Curves.

It will be seen that at the foot of each column in the Tables I to VI, an "adopted" value is given. This value is not the arithmetic mean of the experimental figures, but the value deemed to be the best after considering all the circumstances, giving great weight to the most successful experiments and little or nothing to those of doubtful value. Hence, the factors for the later and better specimens differ only very slightly from the adopted values as shown in the Tables.

With these values a mean curve has been drawn in the following way. First, the band for each separate specimen of protein, that is the part of the curve covered by the "difference" or "amplitude" values was divided at the proper extinction coefficients into ten equal parts and the points of division were designated "position 0" at the extinction coefficient of the head, "1st position" one-tenth of the way down, "2nd position" two-tenths of the way down, and so on until the "10th position" is at the extinction coefficient at the foot of the depression. All the curves were then re-drawn to the scale expressed by the adopted amplitude or "difference" of extinction coefficient.

Next, the wave-length at each "position" on the curve was read from the curve for each specimen, and a value adopted. The adopted values are set out in Table VIII and have been used in plotting the curves (see p. 192).

It should be observed that although the mean curves show points plotted at only about twelve extinction coefficients, and at similar positions for all the specimens, absorption spectra were photographed at 50 or more extinction coefficients, and hence each original curve has a corresponding number of points plotted, similar to those plotted in the curve figured in the first paper.

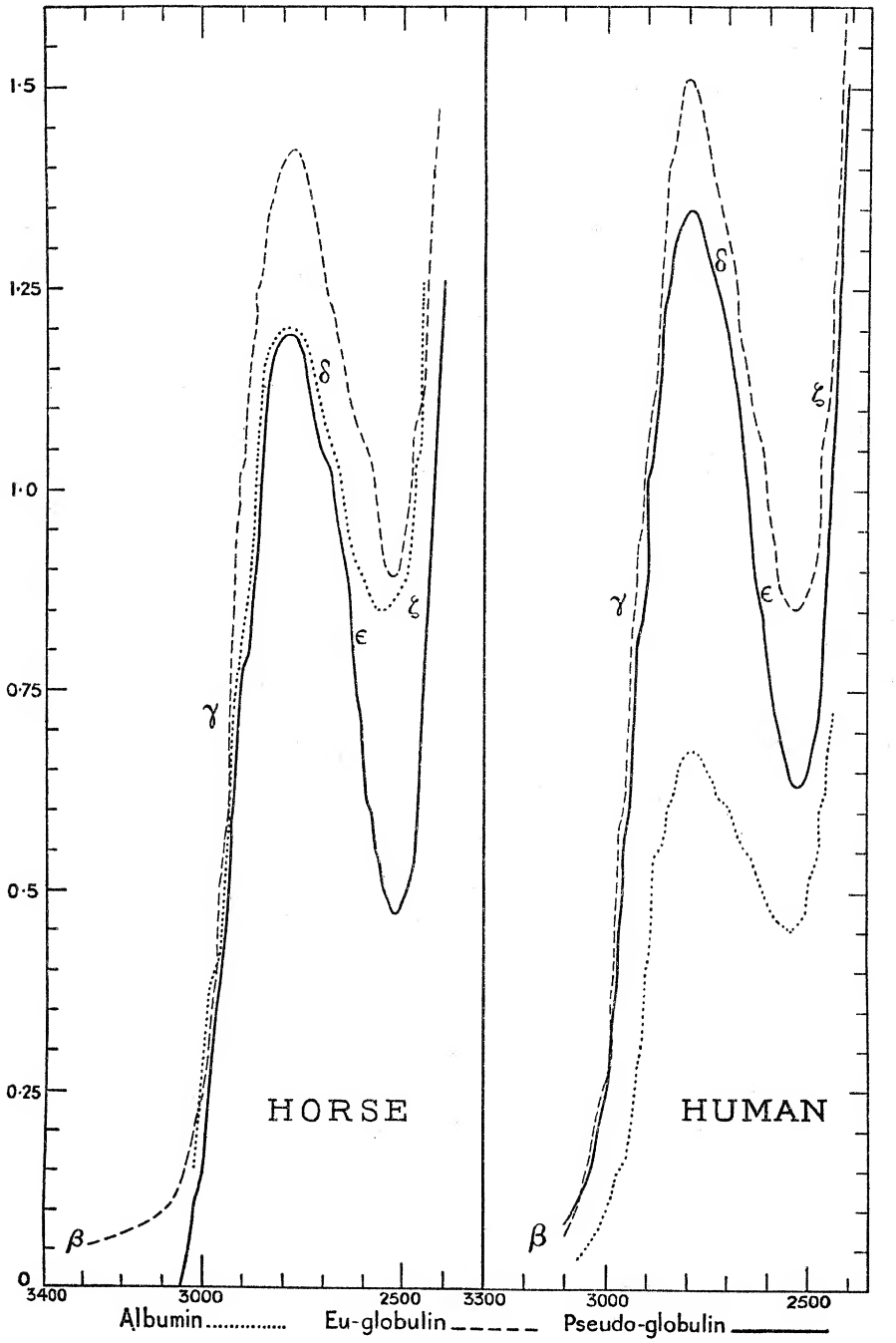
Table VIII.

Protein and variety.	Section.	Position. 0.	1.	2.	4.	6.	8.	9.	10.
		Adopted wave-lengths.							
Pseudo-globulin—									
Horse	γ	2790	2847	2860	2875	2900	2930	2935	2948
	ϵ	2790	2735	2710	2670	2630	2590	2562	2520
	ζ	2410	2415	2420	2430	2445	2460	2475	2520
Human	γ	2790	2845	2858	2864	2895	2929	2935	2943
	ϵ	2790	2744	2710	2686	2635	2595	2572	2521
	ζ	2418	2423	2428	2430	2445	2461	2480	2521
Eu-globulin—									
Horse	γ	2775	2825	2849	2865	2885	2905	2920	2925
	ϵ	2775	2740	2718	2675	2635	2579	2559	2532
	ζ	2420	2435	2437	2443	2460	2480	2490	2532
Human	γ	2795	2830	2850	2864	2880	2905	2920	2930
	ϵ	2795	2760	2730	2680	2648	2605	2578	2530
	ζ	2422	2424	2426	2437	2450	2469	2480	2530
Albumin—									
Horse	γ	2785	2840	2952	2863	2870	2880	2885	2897
	ϵ	2785	2730	2720	2688	2660	2620	2592	2545
	ζ	2456	2457	2460	2465	2475	2490	2508	2545
Human	γ	2783	2815	2825	2850	2871	2880	2882	2885
	ϵ	2783	2755	2730	2685	2645	2610	2575	2540
	ζ	2450	2455	2457	2465	2480	2495	2510	2540

The Characteristics of the Mean Curves.

The curves are divided into Sections α, β, γ, δ, ϵ, ζ, as before (*loc. cit.*). Among the more remarkable characteristics are the curious form of the head, Section δ, and the step-like prominences or "steps" to which reference was made in the first paper. It was hoped that the analysis of the serum into its several proteins would have revealed the origin of these steps by showing them to be irregularities due to imperfect superposition of the bands of two or more proteins, but that expectation has not materialised. On the other hand, these characteristics appear in both the globulins with unerring regularity, and with but little variation, in either magnitude or position. In albumin the "steps" are not so numerous, and they are not much in evidence above the tenth position. Below that two large "steps" are evident.

It is difficult to say whether their positions, that is, their extinction coefficients, are precisely the same for all specimens of a given protein or not. They repeat themselves with sufficient regularity to suggest that the positions may be definite approximately as shown; but on the other hand, the variation is too great to attribute it to experimental conditions. The general conclusion



is that the protein varies, since not only do the positions change more or less, but also sometimes the number of steps in a certain section.

Section β has been developed in only a few cases. The most notable observation is that the absorption in this region is usually very small. The curves and especially the spectrum photographs demonstrate in several cases that the maximum extinction coefficient is considerably less than 0·01. It is certainly very much less with the proteins than it is with serum.

Section γ presents no peculiarities, save only that the sweep is broken by four or five "steps," especially by a prominent one not far from extinction coefficient 0·25.

Section δ is found to characterise all the proteins more or less, although not always in so pronounced a manner as many sera. The mean curve does not exhibit this quite so well as the individual curves do, as the construction of the mean curve has a smoothing-out effect. With human eu-globulin, the head of the curve is narrowed from both sides.

Section ϵ is usually the most irregular in form for any given specimen, and it is here that there is most disturbance in the wave-length. This is partly, but not wholly, accounted for by the "steps."

Section ζ defines the limit of the general absorption, and shows very little variation. Only slight "steps" occur occasionally. The most notable feature is that it bends sharply towards the red at the bottom where it joins the Section ϵ.

Summary.

1. The primary object of the investigation was to ascertain the contribution made by each protein constituent of serum to the ultra-violet absorption spectrum curve of blood serum.

2. It has been shown that the absorption curve of pseudo-globulin is constant, and is the same for both the horse and human varieties.

3. The absorption curve for eu-globulin differs considerably from that for pseudo-globulin in extinction coefficients, but not in general form. This favours the view that the differences between pseudo-globulin and eu-globulin do not result from differences in the structure of the chemical molecule.

4. The absoption curves for the horse and human varieties of albumin have been shown to be the same, except for a constant ratio in their magnitudes, and this difference may be due to the physical, or possibly chemical, association of an aggregate, possessing little or no selective absorptive power—for example, an aliphatic amino-acid or a polypeptide—with the principal or absorbing aggregate.

5. The close similarity in form of all the curves when corrected to a common amplitude and the fact that the amplitudes are nearly all simple multiples of a common factor, point to similarity of constitution amongst these proteins and to a variable "concentration" of the active group.

6. Comparisons between the absorptions of the proteins of human serum reveal absorption bands for the horse somewhat greater in dimensions than those for the human.

7. The optical properties of the proteins of serum have been investigated with fairly satisfactory results.

8. Processes for the separation and purification of the proteins have been elaborated.

The Colouring Matter of Red Roses.

By Geoffrey Currey.

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An examination of the petals of the red rose "George Dickson," has shown that the anthocyan pigment contained therein is the cyanidin glucoside, cyanin. It is present to the extent of about 9–10 per cent. by weight of the dried petals, and exists in the petals as an oxonium salt (*i.e.*, in combination with a plant acid). A yellow glucoside sap-pigment also occurs in the same flowers, but beyond the fact that it has been shown to be capable of producing an anthocyan, by reduction, and that it is not a glucoside of the flavonol myricetin, it has not been further identified, on account of the small quantity present. Further work may show it to be a glucoside of quercetin, and corroborate the work of Dr. Everest,* on the purple-black viola, in which it was shown that an anthocyan ("violanin") and the flavonol glucoside from which it could be produced, by reduction (a glucoside of myricetin), are present, side by side, in the same flowers. This would be additional evidence in favour of the hypothesis that "anthocyans are produced, in nature, by the reduction of the flavonols." It is interesting to note that this rose, grown in Australia, contains the same colouring matter as was isolated by Willstätter and Nolan† from the rose known as "Rosa Gallica," grown in Europe, and shows how widely these colouring matters are distributed in nature.

The rose "George Dickson" was chosen for this investigation on account of its deep red colour, which would indicate a fairly large percentage of the anthocyan pigment. The flowers from which the petals were gathered were grown by Mr. G. Knight, at his nursery, Parramatta Road, Homebush, and

* 'Roy. Soc. Proc.,' B, vol. 90, p. 255 (1918).

† 'Annalen,' vol. 408, p. 1 (1915).